REMARKS

Status of the Claims

Claims 1-4 are currently pending in the application. Claims 1-4 stand rejected. Claim 1 has been amended as set forth herein without prejudice or disclaimer. No new matter has been added by way of the present amendments. Specifically, the amendment to claim 1 is supported by the specification at, for instance, page 3. Reconsideration is respectfully requested.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-4 stand rejected under 35 U.S.C. § 112, first paragraph, for failing to comply with the new matter requirement. (*See*, Office Action of December 21, 2006, at page 2, hereinafter, "Office Action"). Applicant traverses the rejection as set forth herein.

The Examiner states that claim 1 recites new matter because the claim recites, in part, "wherein no exogenous feeder cells are present in the culture." (*Id.*). The Examiner states that page 3 of the specification provides support for "nonhuman animal feeder cells." (*Id.*).

Although Applicant does not agree that the phrase "wherein no exogenous feeder cells are present in the culture" represents new matter, claim 1 has been amended herein, without prejudice or disclaimer, to recite, in part, "wherein no non-human animal feeder cells are present in the culture," as suggested by the Examiner. As pointed out by the Examiner, explicit support for this amendment may be found in the specification at, for instance, page 3.

Since no independent reasoning is provided for the rejection of dependent claims 2-4, other than their dependence from independent claim 1, dependent claims 2-4 are also believed to

not recite new matter since they depend from an independent claim that does not recite new matter.

Reconsideration and withdrawal of the written description/new matter rejection of claims 1-4 are respectfully requested.

Rejections Under 35 U.S.C. § 102(b)

Hiroko et al.

Claims 1-4 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Hiroko et al., EP 1331264A1 (hereinafter referred to as "Hiroko et al."). (*See*, Office Action, at page 4). Applicant traverses the rejection as set forth herein.

The Examiner states that Hiroko et al. disclose a method of co-culturing human chondrocytes together with perichondrial cells and further disclose a cartilage therapy material incorporating a chondrocyte mass obtained by the method. (*Id.*). The Examiner further provides that "without the support or definition as to what is encompassed by 'exogenous feeder cells', the perichondrial cells used in the Hiroko reference are not considered to be 'exogenous.'" (*Id.* at page 5).

Although Applicant does not agree that Hiroko et al. anticipate the presently claimed invention, claim 1 has been amended herein, without prejudice or disclaimer, to recite, in part, "wherein no non-human animal feeder cells are present in the culture," as suggested by the Examiner. Thus, claim 1 no longer recites, "exogenous."

The feeder cells used in Hiroko et al. are chondrogenic-stage perichondral cells from a mammalian fetus which is nonhuman in origin, e.g., a 13-day old murine fetus is disclosed. (*See*,

Hiroko et al., at page 2 paragraphs [0008] and [0009]). One of ordinary skill in the art knows that "chondrogenic stage perichondral cells" means that the perichondral cells are in embryogenesis. However, in the method of Hiroko et al., the proliferation ability of the cells used is eliminated before co-culturing and a perichondrium is not formed during the culturing process. Thus the perichondral cells must be obtained from a source that is in embryogenesis. (*See*, *Id.*). On the other hand, cultured "human chondrocytes" are isolated from human cartilage tissue. (*See*, *Id.* at paragraph [0015]). The cells disclosed at paragraph [0015] of Hiroko et al. are neither on their way to growing nor are they from a mammalian fetus. This fact means that the feeder cells are different origin from the chondrocytes to be cultured.

Thus, Hiroko et al. do not disclose human-originated perichondral cells used in a method for cultivating human chondrocytes.

Further, the Examiner states that human auricular cartilage is known to be coated with perichondrium. (*See*, Office Action, at page 5). Thus, the chondrocytes isolated by the disclosed method of Hiroko et al. must essentially be coated with the perichondrium, according to the Examiner, and therefore co-cultured together with the perichondrium of the cartilage from which it was isolated. (*Id.*). But, the Examiner misunderstands the Hiroko et al. disclosure, as further explained below. That is, Applicant believes that the Examiner's interpretation of the disclosure is scientifically inaccurate.

The Hiroko et al. method co-cultures chondrocytes together with nonhuman origin feeder cells which are prepared as follows:

A sample of cartilage is minced and cultured with trypsin, then with type II collagenase, and the resulting culture medium is filtered with a 100-μm filter to isolate the chondrocytes. (*See*, Hiroko et al., at page 8, paragraph [0015] and page 5, paragraphs [0033] and [0035]).

In this process, perichondrium is removed from the sample of cartilage by cutting. Type II collagenase acts to break up the sample of cartilage into pieces, i.e., chondrocytes which are smaller than 100 μm. However, perichondrium, even if it remained in the sample, is not broken up by type II collagenase because perichondrium does not contain type II collagen. Perichondrium contains type I collagen. (*See*, Gartner et al., "Color Textbook of Histology," 3rd Ed., Saunders Elsevier, 2007, page 132, attached hereto as Exhibit B).

Therefore, filtration enables isolation of chondrocytes to be cultivated, which can pass through the 100 μm filter, from perichondrium, which, even if it remained, cannot pass through the 100 μm filter.

On the other hand, in the method of the present invention, a sample of human cartilage tissue having perichondrium (specification, at page 8, lines 5 to 4 from the bottom) is diced or minced and cultured with type II collagenase, and the resulting culture medium is centrifuged, not filtered (specification, page 9, lines 2 to 11 and page 12 to 13, Example 1). Thus, the resulting precipitate contains both chondrocytes and perichondrium or fragments thereof.

Therefore, since Hiroko et al. do not disclose each and every element as recited in the presently claimed invention, Hiroko et al. cannot anticipate the presently claimed invention. "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." (*See*, *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 U.S.P.Q.2d 1051, 1053 (Fed. Cir. 1987)).

Dependent claims 2-4 are not anticipated as, *inter alia*, depending from a non-anticipated base claim, claim 1.

Reconsideration and withdrawal of the anticipation rejection of claims 1-4 based on the Hiroko et al. reference are respectfully requested.

Klein-Nulend et al.

Claims 1 and 2 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Klein-Nulend et al., *Tissue Engineering*, 4(3):305-313, 1998 (hereinafter referred to as "Klein-Nulend et al."). (*See*, Office Action, at page 5). Applicant traverses the rejection as set forth herein.

The Examiner states that Klein-Nulend et al. disclose culturing human auricular perichondrium containing chondrocytes, wherein no exogenous feeder cells are present in culture. (*Id.* at page 6). However, again, Applicant respectfully disagrees and believes the Examiner's interpretation of the disclosure of Klein-Nulend et al. is scientifically inaccurate, as explained below.

Klein-Nulend et al. disclose the differentiation of progenitor cells contained in human articular perichondrium. (*See*, Klein-Nulend et al. at page 305 and Abstract, lines 1 to 5). Klein-Nulend et al. also disclose that rhOP-1 stimulates differentiation of cartilage from perichondrium tissue. (*See*, *Id.*, at line 15 of Abstract). Thus, in the method of Klein-Nulend et al., human articular perichondrium is not cultured with chondrocytes. In fact, as disclosed in the Materials and Methods section of Klein-Nulend et al., at pages 306 to 307 and Fig. 1, just morsalized perichondrium tissue *per se* is cultured with rhOP-1, without treating with any enzyme, such as trypsin, collagenase, and confirms cartilage formation by assay.

On the other hand, the method of the present invention is based on proliferation of chondrocytes supported by autologous perichondrium. This was discovered for the first time by the present Inventor.

Thus, Klein-Nulend et al. and the present invention are based on completely different principals.

Therefore, since Klein-Nulend et al. do not disclose each and every element as recited in the presently claimed invention, Klein-Nulend et al. cannot anticipate the presently claimed invention. (*See*, *Verdegaal Bros.* 2 U.S.P.Q.2d at 1053).

Dependent claim 2 is not anticipated as, *inter alia*, depending from a non-anticipated base claim, claim 1.

Reconsideration and withdrawal of the anticipation rejection of claims 1 and 2 based on the Klein-Nulend et al. reference are respectfully requested.

Van Osch et al.

Claims 1-4 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Van Osch et al., *Plastics and Reconstructive Surgery*, 2001 (hereinafter referred to as "Van Osch et al."). (*See*, Office Action, at page 6). Applicant traverses the rejection as set forth herein.

The Examiner states that Van Osch et al. disclose "human auricular cartilage and culturing the isolated chondrocytes in a monolayer for 3-4 passages." (*Id.*). The Examiner admits that Van Osch et al. do not disclose that the chondrocytes are co-cultured with pericondrium, but states that human auricular cartilage is known to be coated with pericondrium.

(*Id.* at pages 6-7). However, again, Applicant respectfully disagrees and believes the Examiner's interpretation of the disclosure of Van Osch et al. is scientifically inaccurate, as explained below.

Difference of Process

In the method of Van Osch at al., chondrocytes to be cultured are prepared as follows: A sampled cartilage is sliced and incubated with pronase E, then with collagenase B (type II collagenase), and the resulting medium is filtered with a 100 μm filter to isolate the chondrocytes. (*See*, Van Osch et al., at page 434, left column, line 4 from the bottom to right column, line 7). Thus, pronase E and collagenase B break up the sampled cartilage into pieces, and filtration enables to removal of undigested parts and allows cultivation of the isolated chondrocytes.

On the other hand, in the method of the present invention, a sampled human cartilage tissue having perichondrium (specification, at page 8, lines 5 to 4 from the bottom) is diced or minced and cultured with type II collagenase, and the resulting culture medium is centrifuged, not filtered (specification, at page 9, lines 2 to 11 and page 12 to 13, Example 1). Thus, the resulting precipitate contains both chondrocytes and perichondrium or fragments thereof.

Difference of Multiplication

Van Osch et al. disclose that "Human chondrocytes were often more difficult to culture. The attachment of human cells to the flask was delayed compared with that of rabbit cells and multiplication was much slower." (*See*, Van Osch et al., at page 435, right column, lines 15 to 11 from the bottom).

In contrast, in the method of the present invention, the cell count on the fourth subculture increased about 1000 times, compared with the cell count at the initiation of the subculture. (*See*, specification at page 13, lines 6 to 4 from the bottom).

Difference of production of Collagen type II which is a molecular marker of cartilage tissue

Van Osch et al. disclose that "Human cells cultured in serum-containing medium rarely produced collagen type II (Table I). Replacing serum with IGF and TGF-β increased the number of cells positive for collagen type II, although this number never reached above 10 percent, thus indicating a low degree of redifferentiation." (*See*, Van Osch et al., at page 436, right column, lines 15 to 21).

On the other hand, the products obtained by the method of the present invention were immunologically stained for type II collagen. Doing so indicated that the product was a cartilage-specific matrix. (*See*, specification, at page 14, lines 5 to 9).

Therefore, based on the method of Van Osch at al, it is hardly possible that chondrocytes are co-cultured together with the perichondrium. Thus, Van Osch et al. do not disclose the method of the present invention.

Since Van Osch et al. do not disclose each and every element as recited in the presently claimed invention, Van Osch et al. cannot anticipate the presently claimed invention. (*See*, *Verdegaal Bros.* 2 U.S.P.Q.2d at 1053).

Dependent claims 2-4 are not anticipated as, *inter alia*, depending from a non-anticipated base claim, claim 1.

Reconsideration and withdrawal of the anticipation rejection of claims 1-4 based on the Van Osch et al. reference are respectfully requested.

Larson et al.

Claim 1 stands rejected under 35 U.S.C. § 102(b) as being anticipated by Larson et al., *Matrix Biology*, 2002 (hereinafter referred to as "Larson et al."). (*See*, Office Action, at page 7). Applicant traverses the rejection as set forth herein.

The Examiner states that Larson et al. disclose "producing human chondrocytes by co-culturing chondrocytes with their pericellular matrix attached and no exogenous feeder cells were added to the culture." (*Id.*).

Larson at al. disclose a culture of articular cartilage obtained from human knee. (*See*, Larson et al., at Abstract and at page 350, right column, "2.1 Cell culture"). Although articular cartilage has pericellular matrix (Abstract), articular cartilage has no perichondrium. (*See*, Hirotani, "Standard Orthopaedics," 3rd Ed., Terayama et al., Eds., 1998, page 27, attached hereto as Exhibit A). Additionally, pericellular matrix is not perichondrium. Pericellular matrix contains only matrix components near chondrocytes in cartilage, including type II collagen, but not cellular components, including chondrocytes. On the other hand, perichondrium contains not only matrix components, including type I collagen and fibronectin, but also cellular components including flattened cells in the perichondrium. (*See*, Exhibit B, at page 131).

Thus, Larson et al. do not disclose all the limitations of the presently claimed method. Therefore, Larson et al. cannot anticipate the presently claimed invention. (*See*, *Verdegaal Bros.* 2 U.S.P.Q.2d at 1053).

Dependent claims 2-4 are not anticipated as, *inter alia*, depending from a non-anticipated base claim, claim 1.

Reconsideration and withdrawal of the anticipation rejection of claims 1-4 based on the Larson et al. reference are respectfully requested.

ENTRY OF AMENDMENTS

The amendment to claim 1 should be entered by the Examiner because the amendment is supported by the as-filed specification, as pointed out by the Examiner, and does not add any new matter to the application. Additionally, the amendment should be entered since it complies with requirements as to form, and places the application in condition for allowance. Further, the amendment does not raise new issues or require a further search since the amendment is supported by the as-filed specification. Finally, if the Examiner determines that the amendment does not place the application in condition for allowance, entry is respectfully requested since it likely removes issues for appeal.

CONCLUSION

If the Examiner has any questions or comments, please contact Thomas J. Siepmann, Ph.D., Registration No 57,374, at the offices of Birch, Stewart, Kolasch & Birch, LLP.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to our Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under § 1.17; particularly, extension of time fees.

Dated: April 23, 2007

Respectfully submitted,

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Attachments: Exhibit A - Hirotani, "Standard Orthopaedics," 3rd Ed., Terayama et al., Eds. 1998, page 27
Exhibit B - Gartner et al., "Color Textbook of Histology," 3rd Ed., Saunders Elsevier, 2007, page 132

STANDARD ORTHOPAEDICS

標準整形外科学

Hyojun Seikeigekagaku,

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（執筆順）

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目　次

Contents

図 1-20　関節の模式図

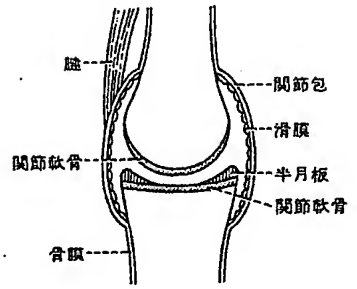
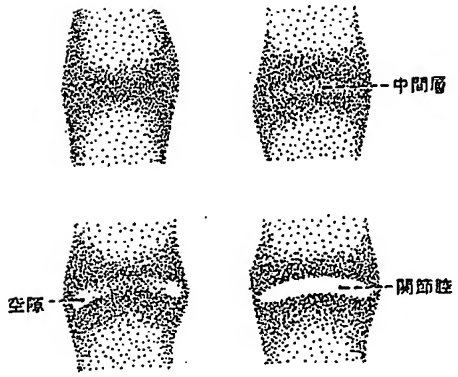


図 1-21　関節腔の形成



周囲の靱帯も関節腔周囲の靱帯から分化したものである。

2. 関節軟骨
2. Articular Cartilage

a. 関節軟骨の構造
a. Structure of Articular Cartilage

滑膜関節は一般に硝子軟骨 hyaline cartilage からできており，その厚さは個体の体重に相関するといわれる。ヒトの膝関節や股関節では 2～4 mm である。成熟した関節軟骨は神経，血管，リンパ管を欠き，滑液によって栄養される。

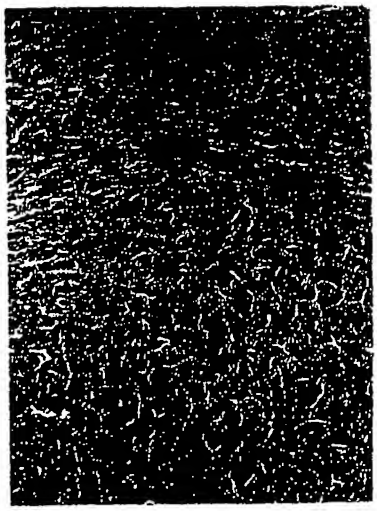
1) 関節表面の構造 articular surface

骨膜，軟骨膜，その他の膜様構造をみない。肉眼的には関節表面はきわめて平滑であるが，走査電子顕微鏡で観察すると非常に凹凸不整である(図 1-22)。すなわち関節の表面には高さ 0.4～0.5 mm のうねり (undulation) があって，さらにそのうねりには 20～30 μ の深さの凹み (pit, depression) が多数みられる。このものは軟骨細胞窩に一致すると考えられている。これらの凹みは潤滑を説明するのに好都合である。

2) 軟骨細胞

Membrane like structure, such as periosteum,

図 1-22　関節軟骨の走査電顕像 (×400)



関節軟骨における軟骨細胞の密度はきわめて低い。成熟した関節軟骨は軟骨細胞の形態，配列や基質の状態から，次の 4 層に分けられる (図 1-23)。

① tangential (gliding) zone: 最表層で扁平な線維芽細胞様の軟骨細胞が関節表面に平行にならび，基質はプロテオグリカン多糖にきわめて乏しい。

② transitional (intermediate) zone: やや楕円形の軟骨細胞が不規則に配列し，プロテオグリカン多糖を組織化学的に基質に証明する。

③ radial zone: 円形の軟骨細胞が関節表面に

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